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Lipidomics Technologies at the End of the First Decade and the Beginning of the Next[1–3]

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ABSTRACT

The lipidome is composed of all of the biomolecules defined as lipids, which encompass compounds of amazing structural diversity and complexity. It has been ~1 decade since the study of "lipidomics" was begun in earnest, and the technologies and tools for data analysis have advanced considerably over this period. This workshop summarized the scope of the lipidome and technologies for its analysis, lipidomics databases and other online tools, and examples of the application of lipidomics to nutritional research. The slides from the workshop, online lipidomics tools, and databases are available at http://www.lipidmaps.org. *Adv. Nutr. 4: 565–567, 2013.*

Introduction

The lipidome is composed of all of the biomolecules defined as lipids, which have been grouped into 8 categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides (1). Compounds with such widely different structures and biophysical properties are difficult to analyze in a global (i.e., "omic") context, but advances in analytical technologies, especially MS, have allowed the term "lipidomics" to first appear in PubMed 1 decade ago (2). That year also saw the beginning of the Lipid Metabolites and Pathways Strategy (LIPID MAPS) Consortium (3), funded by a large-scale collaborative research grant (U54, or "Glue Grant") from the NIH National Institute of General Medical Sciences. This workshop summarized the current perception of the scope of the lipidome, state-of-the-art lipidomic analysis techniques and data analysis tools (including those available on the LIPID MAPS Web site) (4), and examples of research findings that display how lipidomic analysis influences basic and translational science and medicine.

Perception of the Lipidome

Dr. Dennis noted that LIPID MAPS faced a daunting challenge in developing tools for lipidomics because its building blocks have much greater variability than those of genomics and proteomics, with estimates of the number of structural variations in lipids to be $>10^5$ (5). The analytical strategy followed by LIPID MAPS is called comprehensive lipidomic analysis by separation simplification (6), which refers to use of methods that address the structural and biophysical diversity of the lipid categories by optimization of the extraction, chromatographic separations, and MS protocols to obtain quantitative information about the compounds in each lipid category. The analytical methods devised by the LIPID MAPS cores have been described in publications from each of the cores, specialized volumes of *Methods in Enzymology* (7) [all the LIPID MAPS methods in reference 7 can be downloaded as a single PDF file (8)], and *Biochimica et Biophysica Acta* (9). Essential for these methods has been the development of LIPID MAPS standards in collaboration with Avanti Polar Lipids for most of the categories and Cayman Chemicals for eicosanoids.

Applications of these technologies were presented for analysis of the mouse lipidome (10) upon activation by a defined Toll-like receptor 4 agonist, Kdo_2-lipid A, developed by LIPID MAPS (11), and human plasma (12). The power of combining analysis of transcriptomic, proteomic, and lipidomic data was illustrated for eicosanoid metabolism (13), and the impact of supplementation with different PUFAs (arachidonic acid, EPA, or DHA) on the metabolite profiles

[1] This article is a summary of the symposium "Lipidomics Technologies at the End of the First Decade and the Beginning of the Next" held April 23, 2013 at the ASN Scientific Sessions and Annual Meeting at Experimental Biology 2013 in Boston, MA. The symposium was sponsored by the American Society for Nutrition and supported in part by NIH grant GM069338 (LIPID MAPS).

[2] The slides from the presentations can be seen on the LIPID MAPS Web site.

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was discussed (14). More than 350 papers have been published by LIPID MAPS during its decade of NIH funding.

Lipid Analysis by Multiple "Omic" MS Platforms

Dr. McDonald summarized the effort, expense, and specialized expertise that is required for the structure-specific, quantitative lipidomic analysis exemplified by these LIPID MAPS coordinated studies (10,12), which typically used more than a dozen different extraction conditions to optimize recovery of the different lipid classes, 3 gas and 14 liquid chromatography (LC) separations combined with several MS platforms, and hundreds of internal standards. The power of this approach has been seen it its ability to quantify nearly 600 different molecular species of lipids in human plasma (12), including sterols (15) that range in amounts by 6 orders of magnitude, from grams per liter for cholesterol to micrograms per liter for some trace oxysterols.

Other approaches provide quicker profiling by "shotgun lipidomics" (16), and broad-scale, targeted, and untargeted LC/MS "omics" methods also include some lipids among the ions detected (see references 17 and 18 for examples). These methods have been useful in finding new connections between lipids and disease, but low-abundance and isomeric/isobaric lipids can be missed. The future of lipidomics analysis will likely involve a combination of technologies based on the goals of the analysis, with key players being *1*) chromatograhic-based quantitative analysis with greater column resolving power and efficiency as well as tandem MS and MS^n analysis of higher acquisition speed and sensitivity; *2*) infusion-based analysis with high resolution, mass accuracy, and linearity, with offline LC followed by infusion-based MS; *3*) ion-mobility MS for resolution of compounds that are difficult to resolve by standard LC and MS methods; and *4*) incorporation of tissue-imaging MS into the portfolio of methods available to lipid researchers. For all of these techniques, accessibility to standards and data analysis software will be essential to allow the data to be accurate and interpretable.

Lipidomics Databases and Other Online Tools

Dr. Fahy presented an overview of the LIPID MAPS bioinformatics tools and data (19,20), which are accessible through the Web site (4) (the Web site also has links to tutorials on how to use the tools and databases), and discussed their use to develop a systems biology of lipids (21). The tools on this Web site meet the need for high-quality bioinformatics to manage and integrate experimental data at multiple levels (22): *1*) definition of lipid classification and ontologies, *2*) relational database design, *3*) capture and automated pipelining of experimental data, *4*) efficient management of metadata, *5*) development of lipid-centric search tools, *6*) analysis and visual display of results, and *7*) integration of the lipid knowledge base into biochemical pathways and interactive maps.

The databases can be queried by text, mass, formula, classification, and other criteria, or most simply by "quick search" to obtain information about not only lipids but also MS standards, the lipid proteome database, and pathways. In addition to building these databases from known compounds, LIPID MAPS has developed a software package called LipidMapsTools for the template-based combinatorial enumeration of virtual compound libraries for lipids (23).

Many of the tools and databases on the LIPID MAPS Web site have already gained widespread use, such as the classification system, tools for drawing lipid structures (which have become standard for structures published in the *Journal of Lipid Research*, *Biochimica et Biophysica Acta*, and other journals), and identification of lipids by mass-to-charge ratio and fragmentation (and vice versa). The LIPID MAPS Web site (4) with these tools will be maintained with funding from the NIH after the end of the consortium.

Lipidomics Comes of Age in Nutrition and Other Translational Sciences

Dr. Merrill noted that nutritional scientists were some of the first to publish data that would today be defined as "omic" as they sought to know all of the compounds found in food and the body to be able to relate diet to health. Their "omic"-scale contributions can be found in not only the research literature but also the extensive databases maintained by the U.S. Department of Agriculture (such as the USDA National Nutrient Database for Standard Reference), the CDC (as part of the National Health and Nutrition Examination Survey), and others. These databases have many uses because they are well established and easy to access, but they have the disadvantages—from an "omics" viewpoint—of covering only known nutrients and being difficult to individualize. In 2002 *The Journal of Nutrition* served as the forum for an insightful opinion piece by German et al. (24) that discussed how technological developments were beginning to allow nutritionists to obtain metabolomics data that could be used in combination with data from other sources, including genomic analysis, for individual metabolic assessment. The past decade has seen a rapid growth in nutritional research using lipidomics as discussed in several reviews (25–29), including one using the term "foodomics" (28) and one for fecal lipidomics (29), and as a factor in design of new crops (30).

A special challenge for nutritional lipidomics is that lipids made by organisms that are consumed as food have tremendous structural variation. This variation raises many questions, such as how are dietary lipids digested, absorbed, and/or excreted and do they affect the intestine directly or through altering intestinal microflora? When exogenous lipids are taken up by the body, how are they transported, metabolized, and incorporated into tissues? Do exogenously obtained lipids have special functions or bioactivities? How do exogenous lipids affect the metabolism and functions of endogenous lipids? In addition to these considerations, a large proportion of the other components of the diet and an individual's physiologic factors (e.g., genetics, age, physical activity) affect lipid metabolism.

Sphingolipid research represents one of the fields in which many surprises have been encountered as each of these questions has been addressed with lipidomics approaches, including the discovery that dietary sphingolipids are able to affect physiology and disease (31). Indeed, one surprise was the recent discovery of a new category of backbone sphingoid bases (1-deoxysphinganines) that are made when alanine is utilized instead of serine in the first step of the pathway. These bases are also present in food and have been shown to affect health (31).

Perspectives for the Future

Lipidomics technologies have come a long way in the past decade and have firmly established the value of lipidomic analysis. One might conclude that the field has grown to the level of sophistication at which inclusion of lipidomic analysis is mandatory for studies that claim to be a comprehensive evaluation of a biological system or process. This growth in the field also may have reached the critical mass that prompts instrument manufacturers to develop more robust, faster, simpler, and (one hopes) cheaper instruments to accommodate a rapidly expanding number of users.

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